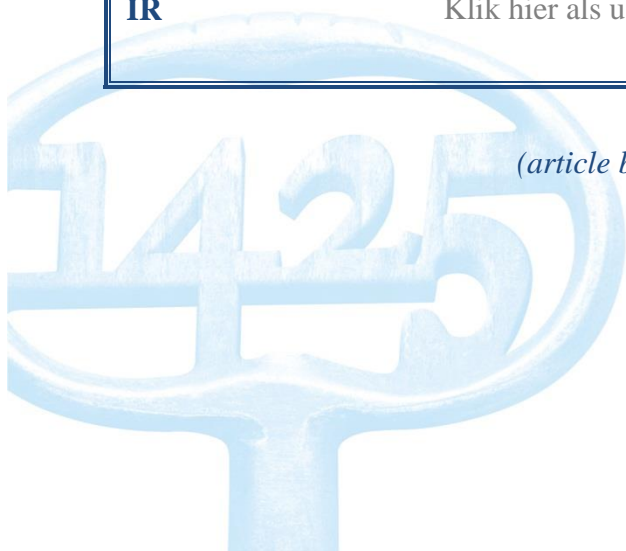




Citation	<p>Francesca Di Bartolomeo and Wim Van den Ende</p> <p>Fructose and fructans: Opposite effects on health?</p> <p>Plant Foods for Human Nutrition vol:70 issue:3 pages:227-237</p>
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Abstract

Fructans are fructose-based oligo- and polysaccharides of natural origin. Fructan and fructose species are sometimes confused by the great public, although they clearly have different biochemical and physiological properties. This review discusses aspects of the use of fructose and fructans in foods in the context of human health, with possible differential effects on cellular autophagy in cells of the human body. Although there are uncertainties on the daily levels of ingested fructose to be considered harmful to human health, there is an emerging consensus on the benefits of the use of fructans in functional foods, sustaining health via direct immunomodulatory and antioxidant effects or through indirect, prebiotic mechanisms.

Abbreviations. AGEs: advanced glycation end products; ATP III: Adult Treatment Panel III; ChREBP: carbohydrate-responsive element binding protein; CVD: cardiovascular diseases; DHAP: dihydroxyacetone phosphate; eNOS: endothelial nitric oxide synthase; ER: endoplasmic reticulum; FA: fatty acid; FOS: fructo-oligosaccharides; GGT: gamma glutamyl transferase; GI: glycemic index; GLUT: glucose transporter; Gr43a: gustatory receptor 43a; HFCS: high-fructose corn syrup; IRS: insulin receptor substrate; JNK: c-jun N-terminal kinase; NAFLD: non-alcoholic fatty liver disease; PFK-1: phosphofructokinase 1; PKC: protein kinase C; RBP-4: retinol binding protein-4; ROS: reactive oxygen species; SCFAs: short chain fatty acids; SREBP-1: sterol regulatory element-binding protein 1; TG: triglyceride; VLDL: very-low-density lipoproteins

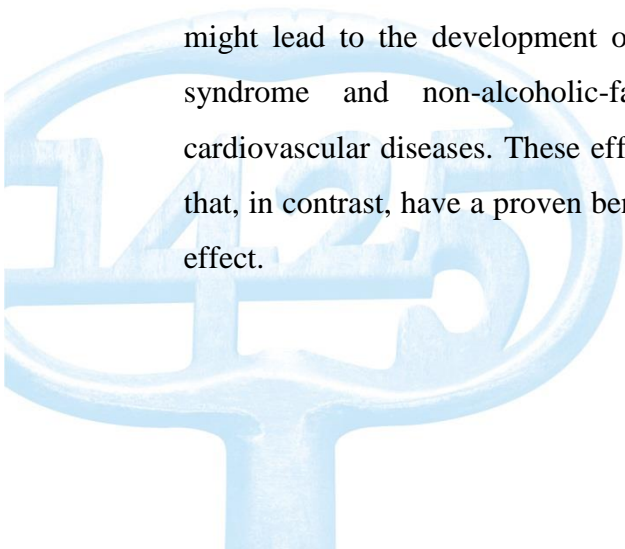
Keywords. Diabetes, health, food, fructan, fructose, prebiotics, oxidative stress

Introduction

Sweeteners are known since the early twentieth century when saccharin, a molecule 300-500 times sweeter than table sugar and developed by Constantin Fahlberg in 1879, became popular during the First World War [1]. Since then,

many other substances were introduced into the world market with the purpose to sweeten foods and beverages. Contrary to table sugar (saccharose or sucrose, a disaccharide consisting of glucose and fructose), they have a low or zero caloric (Kcal) value and minimal doses are required to obtain the same sweetening effect. Some of these sweeteners, currently found in any supermarket or added to snacks and soft drinks labelled as "light", have a natural origin (e.g. fructose, stevioside, xylitol), but most of them are synthetic (e.g. sucralose, saccharin, aspartame). Although short chain fructans, natural fructose-based oligosaccharides (FOS), have sweetening properties as well, they are less sweet than fructose and sucrose [2] and therefore they are not particularly used with the purpose to sweeten foods and beverages. Combinations of FOS and for instance stevioside [3] may lead to the desired reduced caloric intake and sweetening effects. Fructans are health improving compounds used as prebiotics and fat replacers in various foods, but they are interesting in terms of non-food applications as well [4].

The difference between fructose and fructans is not always clear to the general public, and general misconceptions exist on their terminology and physiological effects. While at the end of the 70s fructose was suggested as useful addition in diets of diabetic patients, now it becomes clear that higher doses of fructose might lead to the development of pathological conditions such as metabolic syndrome and non-alcoholic-fatty-liver, in turn increasing risks on cardiovascular diseases. These effects are emphasized comparing it to fructans that, in contrast, have a proven beneficial prebiotic action and health improving effect.



Consequences of fructose assumption in the human diet

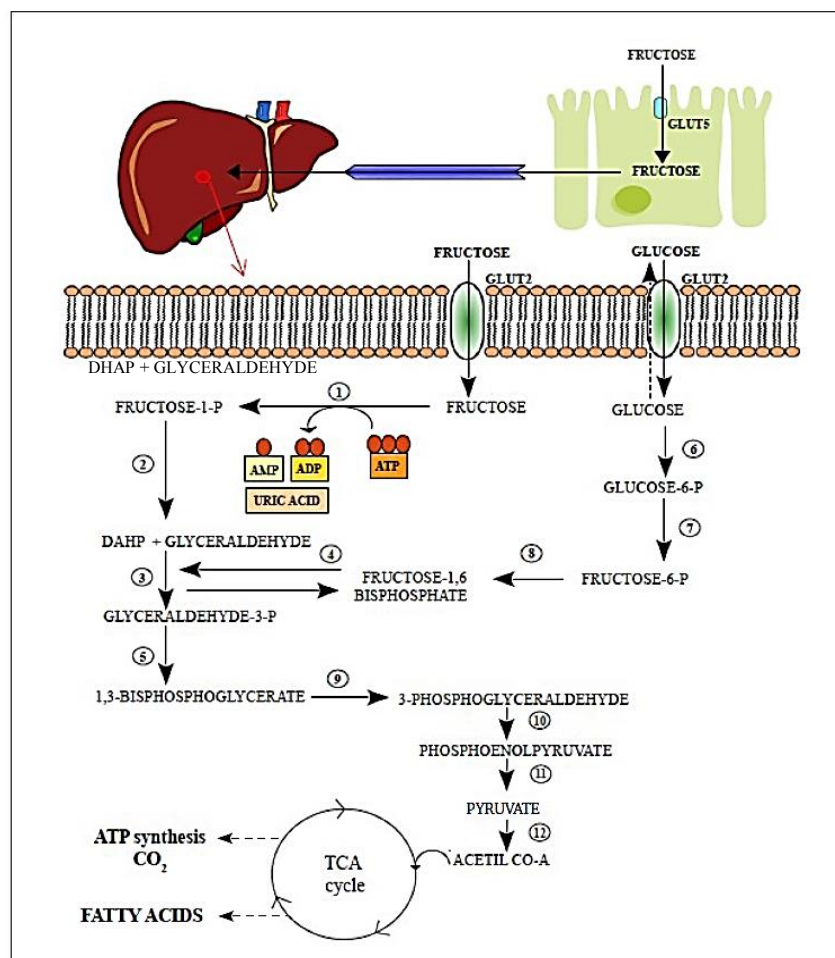
Fructose ($C_6H_{12}O_6$), a hexose monosaccharide, is a ketose-type reducing sugar. Although its molecular formula is identical to the aldose glucose, it is clearly different in many chemical and metabolic facets. By linking glucose and fructose, sucrose is formed and the reducing character is lost. Until the early nineteenth century the human diet has been characterized by the intake of a limited amount of sugar and sweeteners. For a long time the only source of fructose were fruits and honey, in which it is present at lower levels. For instance, the amount of fructose in a medium size raw apple is about 11 g, while the average fructose content in one tablespoon of honey is 9 g. The fructose ingested in this way and in small quantities is beneficial since it improves the glycaemic response after administration of glucose in subjects suffering from type 2 diabetes [5]. It has been established by the American Diabetic Association that in the United States the recommended quota of energy derived from sweeteners should not go beyond 25% of the total energy but this limit is currently exceeded by a quarter of children [6]. From 1970 to 1997, the use of sweeteners has steeply increased. For example, the per capita fructose consumption rose from 64 g/day to 81 g/day. Fructose is mainly taken up in the form of sucrose and high-fructose corn syrup (HFCS), which is composed by either 55% or 42% of fructose [7]. Based on incomplete assumptions, in 1986, fructose was even suggested as a low cost substitute of glucose for diabetic patients [8]. In the same year, Levin et al. proposed that fructose, in contrast, does not stimulate thermogenesis, thus promoting obesity more than glucose [9]. This initial hypothesis on the influence of fructose on the metabolism prompted many other studies on high fructose intake and the onset of metabolic syndrome and other disorders.

According to the Adult Treatment Panel III (ATP III) Report, the metabolic syndrome, also known as X-syndrome, can be defined as a set of clinical manifestations such as abdominal obesity, atherogenic dyslipidemia, hypertension, insulin resistance and glucose intolerance, all predisposing factors to cardiovascular diseases (CVD) [10]. Experiments performed on rhesus monkeys (*Macaca mulatta*), a species closely resembling the human race, demonstrated that a high-fructose diet caused, within a short time period of time, insulin resistance and many features of the metabolic syndrome as central

obesity, dyslipidemia and inflammation. Moreover, a subset of monkeys developed type 2 diabetes [11].

Spotlight on fructose metabolism and the disorders linked to its overconsumption

After its intake from dietary sources, fructose is passively transported across the intestine membranes by GLUT5, a member of the facilitative glucose transporter (GLUT) family, and also an important control point for fructose absorption and distribution to other tissues [12] (Fig. 1).



Fructose ingested with the diet is passively translocated and absorbed within the enterocytes by the transporter GLUT5. Herein a part of it is conveyed by the portal circulation to the liver where the carrier protein GLUT2 allows the passage in hepatocytes. The hepatic enzyme system responsible for the metabolism of fructose and glucose comprises the following catalysts: **1.** Fructokinase **2.** Fructose 1,6-bisphosphate aldolase **3.** Triose-phosphate isomerase (Tim) **4.** Fructose 1,6-bisphosphate aldolase **5.** Glyceraldehyde-3-phosphate dehydrogenase **6.** Hexokinase **7.** Phosphoglucose isomerase **8.** Phosphofructokinase-1 (Pfk-1) **9.** Phosphoglycerate kinase (Pkg) **10.** Enolase **11.** Pyruvate kinase **12.** Pyruvate dehydrogenase complex (Pdc).

It should be noted that in subjects with type 2 diabetes, the level of mRNA and proteins corresponding to the duodenal GLUT5 is up to 3-4 times higher than in non-diabetic subjects [13]. It has been demonstrated in some patients that reducing the state of hyperglycaemia can normalize the up-regulation of a specific transporter. These observations suggest that GLUT5 might be involved in the metabolic perturbations linked to high-fructose nutrition [14]. Once fructose and glucose are absorbed in enterocytes they go directly across the portal circulation which conveys them to the liver, where an active hepatic enzyme system rapidly metabolizes fructose, converting it into glyceraldehyde and dihydroxyacetone phosphate (DHAP), representing the connection point between the fructose and glucose metabolic pathways [15].

One of the distinctive features of fructose metabolism as compared to glucose metabolism is that it does not rely on the activity of phosphofructokinase (PFK-1) (Fig. 1). PFK-1 converts fructose 6-phosphate to fructose 1,6-bisphosphate and is pivotal in the regulation of the glycolytic flow, influencing cell metabolism and systemic metabolic conditions. Given its central role, this enzyme is controlled by the concentration of an high number of metabolites such as AMP, ADP and fructose 2,6-bisphosphate which act as activators and ATP, phosphoenolpyruvate (PEP) and citrate that inhibit PFK-1 [16].

In contrast to glucose, the peptide hormone insulin is unable to influence the hepatic metabolism of fructose and its conversion to triose phosphates. Thus

fructose enters the glycolytic pathway unhindered and generates an excess of energy flux caused by the low K_m of the enzyme fructokinase and the concomitant lack of a negative feedback from ATP or citrate. Direct consequence is a transient depletion of free phosphate and a decrease in the ATP concentration in the hepatocytes. Triose phosphates produced from fructose can subsequently be converted into pyruvate and oxidized to CO_2 and H_2O in the tricarboxylic acid cycle. Alternatively, they might be converted in fatty acids triggering the process of *de novo* lipogenesis [17] (Fig. 1).

Another hypothesis, explaining the propensity of fructose to lead to lipogenesis, states that the very rapid phosphorylation of fructose by fructokinase stimulates triglyceride synthesis via a purine-degrading pathway. Indeed, the generated AMP enters the purine degradation pathway through the activation of AMP deaminase, uric acid production and the generation of mitochondrial oxidants. These oxidants negatively interact with aconitase, which catalyses the conversion of citrate to isocitrate in the Krebs cycle, causing the accumulation of citrate and the stimulation of ATP citrate lyase and fatty-acid synthase, finally leading to the induction of *de novo* lipogenesis [18]. This might explain why hepatic ATP depletion (and impaired ATP recovery), and elevated uric acid levels are considered as risk factors for the development of non-alcoholic fatty liver disease (NAFLD) [19].

Cox et al. (2012) reported that an intake of fructose (corresponding to 25% of energy requirements) for 10 weeks contributes to characteristic features of metabolic syndrome by increasing the amounts of circulating uric acid, the activity of gamma glutamyl transferase (GGT) linked to changes in liver functionality and the production of retinol binding protein-4 (RBP-4) [20]. High values of RBP-4 were also observed in women with NAFLD [21].

An additional hypothesis was launched relating fructose intake with hyperuricemia and the onset of insulin resistance. This hypothesis is based on

the fact that insulin, through the activation of the endothelial enzyme nitric oxide synthase (eNOS), can increase the blood flow to the skeletal muscles, in this way influencing glucose utilization [22]. The eNOS is strongly inhibited by high concentrations of uric acid, which might block the vascular effects of insulin and thus induce a state of insulin-resistance linked to the fructose assumption [23].

At the end of the 70s, one of the reasons behind the erroneous suggestion of administering fructose to diabetic patients, as a valid alternative to glucose, is its low glycemic index (GI). In fact the glycemic index of fructose is 20, a very low value as compared to glucose with a GI of 100. The GI is considered to reflect the capability of a particular carbohydrate to raise the rate of glycaemia, thus, it can be a useful parameter to describe and compare diverse nutrients according to the different induction of plasma glucose production after their ingestion [24]. Furthermore, the GI influences the degree of satiety that an aliment is able to confer because there is a correlation between insulin production and leptin stimulation. According to Klok et al. (2007), leptin is a mediator of the long-term regulation of energy balance, suppressing food intake and thereby inducing weight loss [25]. Since fructose does not stimulate insulin secretion by pancreas β cells, it reduces a leptin level that in turn is not able anymore to reduce the appetite [26]. Based on this view, Shapiro et al. (2008) hypothesized that chronic fructose consumption leads to leptin resistance, which subsequently may promote the development of obesity in response to a high-fat diet [27]. In obese subjects with insulin resistance, consumption of fructose compared with glucose, in the form of soft drinks during meals, has been related with a decreased production of insulin, a leptin weak diurnal activity and an enhanced concentration of triglycerides (TGs). These increases indicate that fructose consumption may aggravate the impaired metabolic profile typical in overweight individuals. Other experiments on rats, instead, show that an acute fructose uptake creates a state of leptin resistance though an enhanced amount of cytokine 3 suppressor and through a decreased serine/threonine phosphorylation of key proteins in leptin signaling. In the liver leptin elicits fat mobilization and oxidation, thus, a fructose-induced leptin resistance might lead to the development of NAFLD [28].

It has already been mentioned that one of the postulated consequences linked to abuse of fructose is the development of type 2 diabetes, which is a condition

often proceeded by a state of insulin resistance [29]. Indeed, a high fructose diet increases glucose and insulin responses to sucrose load, increasing fasting glycaemia, and leading to hepatic insulin resistance in healthy men [30]. The early stages of type 2 diabetes are characterized by a condition of hyperinsulinemia, with a concomitant increase in the levels of C-peptide (a polypeptide derived from pro-insulin in the pancreas). The C-peptide blood test allows distinguishing between diabetes type 1 and 2 in the disease early-stage. A patient with a pancreatic dysfunction and incapable of producing insulin (type 1 diabetes) is also unable to produce the C-peptide since it is a by-product of insulin maturation. On the other hand in the occurrence of type 2 diabetes, as in this case, the level of C-peptide are usually normal or even higher due to a reduced sensitivity of cells to insulin action which in turn might cause a state of insulin resistance. Only at the stage of overt metabolic disorder, the chronic overload of the pancreas trying to compensate for the deficiency at the cellular level, leads to a progressive decay of pancreatic β -cell function and thus insufficient insulin secretion. In a population study on 1999 healthy women, plasma C-peptide concentrations were recorded. Overall, high intakes of fructose and high glycemic foods were associated with a higher C-peptide concentration, suggesting that high fructose intake might play a role in the development of insulin resistance and type 2 diabetes [31]. There is a tight association between lipid metabolism disorders and insulin resistance; indeed an excessive intramyocellular triacylglycerol combined with a reduced lipid turnover may produce toxic lipid-derived metabolites, such as diacylglycerol, fatty acyl CoA, and ceramides which, in the intracellular environment, lead to a higher serine/threonine phosphorylation of the insulin receptor substrate-1 (IRS-1), reducing insulin signaling. An alteration in the post-receptor insulin signaling was proposed in rodents [32].

The administration of high-sucrose diet for three weeks did not alter the number of insulin receptors (IRS-1 and IRS-2), but their activity was affected by an elevated basal phosphoinositid-3-kinase leading to a reduction of their degree of phosphorylation [33]. In a similar experiment, rats were fed for 28 days with a high-fructose diet, and also in this case, as for the high-sucrose diet, a reduction in the IRS 1 and 2 phosphorylation were recorded, both in the liver and in muscles [34]. Samuel et al. (2010) suggested that toxic compounds such as diacylglycerol activate a novel protein kinase C (novel-PKC). The activation of PKCs is notably involved in the regulation of insulin activity through their effect on serine phosphorylation of IRS1 [35]. However, which kinase(s) are involved in the serine phosphorylation of IRS remain(s) unknown. What is known is that the phosphorylation of IRS1 at serine-307 prevents IRS1 from interacting with the insulin receptor. This leads to a consequent decrease in the tyrosine-phosphorylation of the insulin receptor, resulting in increased hepatic glucose production, impaired glucose tolerance and increased fasting glucose and insulin concentrations that may contribute to the development of hyperglycaemia and type 2 diabetes [36] (Fig. 2).

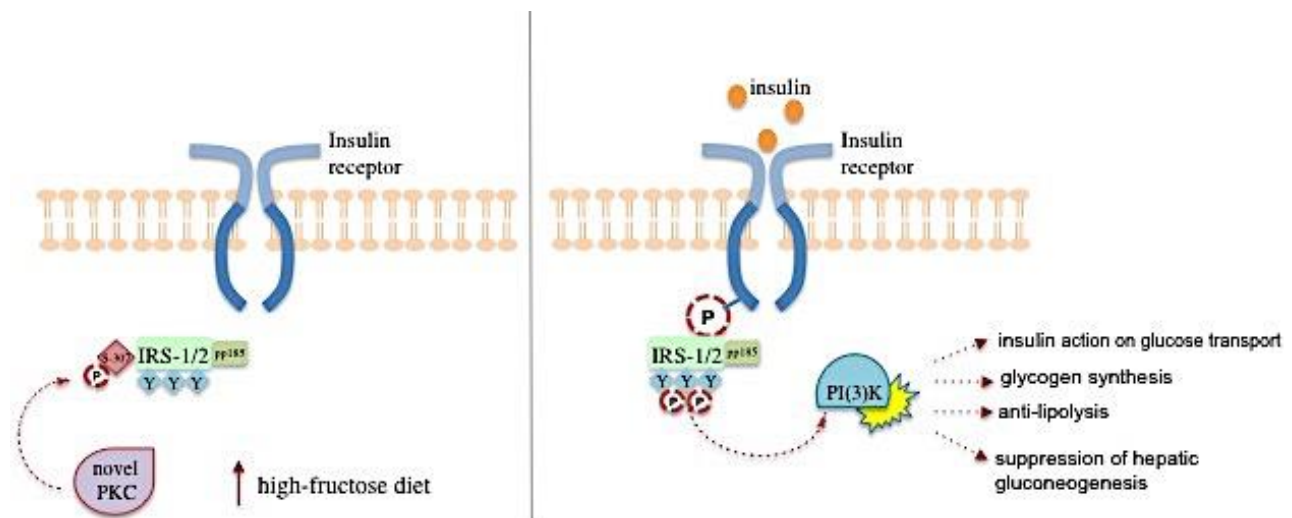


Fig. 2. Effect on high-fructose diet on the activity of insulin receptor substrate 1 and 2

The status of insulin resistance induced by high-fructose administration is correlated with modifications in the early steps of insulin signal transduction. In the regular process insulin triggers the tyrosine kinase activity of its receptor resulting in the tyrosine phosphorylation of pp185, which contains insulin receptor substrates IRS-1 and IRS-2. Feeding animals a high-fructose diet results in insulin resistance, which is not related to the IRS-1 /2 protein levels but it is due to a decreased phosphorylation of the insulin-induced pp185 (IRS-

1/2). Moreover the intracellular accumulation of lipids, which is correlated with a high-fructose diet, activates the novel protein kinases C (novel PKC) with subsequent impairments in insulin signaling caused by the phosphorylation of the serine-307 in IRS1 which prevents its interaction with the insulin receptor.

It has also been found that high doses of fructose administered by intraportal infusions (portal vein fructose concentration >1 mmol/L), trigger a hepatic stress response identified by the activation of c-jun N-terminal kinase (JNK). When the level of hepatic fructose uptake goes beyond the need of glycogen and energy production, mechanisms of adaptive response to stress are established. In these conditions an increased phosphorylation of the mitogen-activated protein kinase kinase-7 (MKK7) has been observed, which is capable of activating JNK that, in turn, associates with c-jun N-terminal kinase-interacting protein-1(JIP1). In the liver the activation of JNK leads to the phosphorylation of serine-307 on IRS-1 [37].

As explained above, high intake of fructose can strongly stimulate lipogenesis. An overload of the lipid-export machinery and the disturbed mitochondrial lipid degradation lead to intrahepatic fat deposition or steatosis [38]. In healthy volunteers, the intake of fructose in high quantities increased plasma total lipoproteins and very-low-density lipoproteins (VLDL). Moreover, fructose may contribute to the biogenesis of both glycerol and the fatty-acyl parts of VLDL-triglycerides [39]. In rats, the *in vivo* administration of [¹⁴C]fructose led to ¹⁴C incorporation in liver lipids [40]. A similar experiment demonstrated the stimulation of hepatic *de novo* lipogenesis after acute fructose uptake in humans, detecting the inclusion of infused ¹³C-labeled acetate into VLDL-palmitate [41]. Further, fructose causes the concomitant inhibition of fatty acid oxidation in the mitochondria of hepatocytes. Fructose-mediated malonyl-CoA increases inhibit the carnitine palmitoyl transferase-1 (CPT- 1), reducing the

translocation of fatty acids into the mitochondria [42]. Fructose has also the ability to stimulate the induction of hepatic lipogenic enzymes, such as the transcription factor sterol regulatory element-binding protein 1 (SREBP-1c), in a process that is totally independent from the action of insulin, the main inducer of hepatic lipogenesis [43].

All these above-mentioned processes are considered as evidence in favor of the hypothesis that dietary fructose might promote the development of NAFLD, which can culminate in hepatic insulin resistance, often the first sign of an increased risk on developing type 2 diabetes [44].

Fructose effects are under debate

Despite the large amount of data on fructose feeding experiments, the issue remains controversial. Perhaps the doses used in some of the above-mentioned experiments were far too high [45]. The long-term effects of moderate, daily fructose intakes (e.g. by drinking a soda per day) remain ambiguous. Some recent researches suggest that fructose consumed in a typical diet, as part of commonly used sweeteners such as sucrose or HFCS, is not able to promote the deposition of ectopic fat in the liver or muscles. Recently White (2013) predicted that fructose intake, in accordance with normal population habits, is not able to alter biochemical outcomes more than any other dietary sugars; and moreover Laughlin (2014) claim that modest level of fructose might acts synergistically with glucose increasing the disposal of a dietary carbohydrate load [46]. Nevertheless, a meta-analysis was conducted with a dosage that resembled the average daily intake in the United States (>50 g/day). Such levels are associated with increased postprandial TG range. A fructose intake >100 g/day has been associated with increased fasting TGs [47]. In a recent study a double-blind, randomized, cross-over trial has been performed in healthy young men by feeding them for three weeks with 600 mL a day of four different sweetened beverages containing medium fructose (MF) at 40 g/day, high fructose (HF), high glucose (HG), and high sucrose (HS) each at 80 g/day. The results showed that fructose, as compared with glucose, is able to alter in a significant way the hepatic insulin sensitivity and lipid metabolism [48]. It can be concluded that these last two examples instantiate the need for further

studies on this topic, rather than using them as a support against the use of fructose in food applications.

Fructans: beneficial for health

Fructans are fructose-based oligo- and polysaccharides containing maximal one glucose unit. They have different and unique properties as compared to their fructose constituents and as compared to glucans (polymers of glucose) [49]. Five main fructan subgroups can be defined on the basis of the position of glucose moieties (internal or end position) and on the glycosidic linkages between their fructosyl residues [50] (Fig. 3). A sixth subgroup was proposed, consisting of mixed neo-type graminans, also termed agavins [51]. The best studied linear inulin-type fructans, typically occurring in Asteracean species, contain fructose units linked through β -2,1 bonds. 1-kestotriose (1-kestose) is the building block of this series. In plants, it is produced by the activity of sucrose:sucrose 1-fructosyl transferase (1-SST), transferring a fructosyl residue from a donor to an acceptor sucrose substrate. Fructan:fructan 1-fructosyl transferase (1-FFT) then polymerizes 1-kestotriose into higher DP inulin-type fructans. Sucrose:fructan 6-fructosyl transferase (6-SFT) preferentially transfers a fructosyl moiety from sucrose to 1-kestotriose, producing 1&6-kestotetraose (also termed bifurcose), the smallest graminan-type of fructan with mixed type of linkages, as typically occurring in cereals. Next, bifurcose can be further extended by 6-SFT and 1-FFT, leading to higher degree of polymerization (DP) graminans. Linear levan-type fructans, as occurring in some fodder grasses, have only β -2,6 fructosyl-fructose bonds and are synthesized by a 6-SFT with intrinsic sucrose:sucrose 6-fructosyl transferase characteristics (a 6-SST/6-SFT) [52]. The enzyme fructan:fructan 6G-fructosyl transferase (6G-FFT)

synthesizes 6G-kestotriose (6G-kestose, neokestose) from 1-kestotriose as donor substrate and sucrose as acceptor substrate. Elongation by 1-FFT and 6-SFT leads to the formation of inulin neoseries and levan neoseries as for instance occurring in asparagus and *Lolium* species, respectively [50] (Fig. 3).

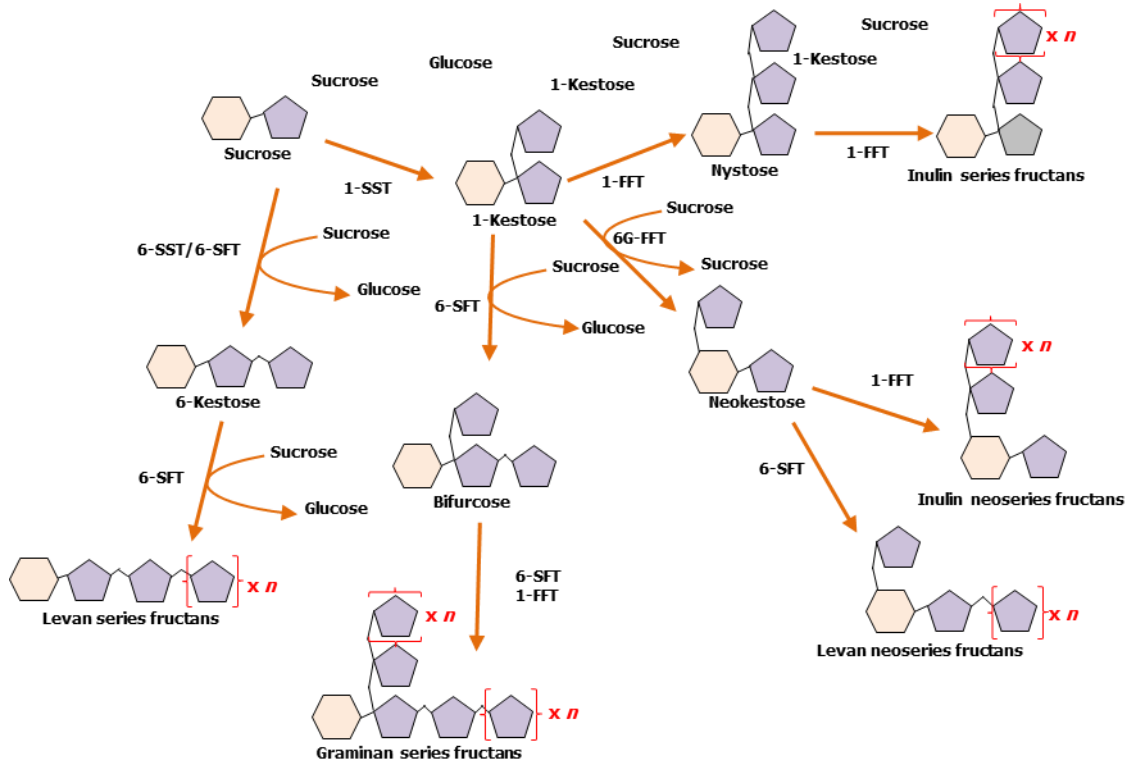


Fig. 3. Plant fructan biosynthesis from sucrose

Five classes can be discerned: inulin- (linear), levan- (linear), graminan- (branched), neo-inulin (branched) and neo-levan type (branched) fructans. The following enzymes are involved: sucrose:sucrose 1-fructosyl transferase (1-SST), a dual function sucrose:sucrose 6-fructosyl transferase/sucrose:fructan 6-fructosyl transferase (6-SST/6-SFT), sucrose:fructan 6-fructosyl transferase (6-SFT), fructan:fructan 6G-fructosyl transferase (6G-FFT) and fructan:fructan 1-fructosyl transferase (1-FFT). The trisaccharides 1-kestose, 6-kestose and neokestose and the trisaccharide bifurcose are building blocks from which higher degree of polymerization (DP) fructans are synthesized.

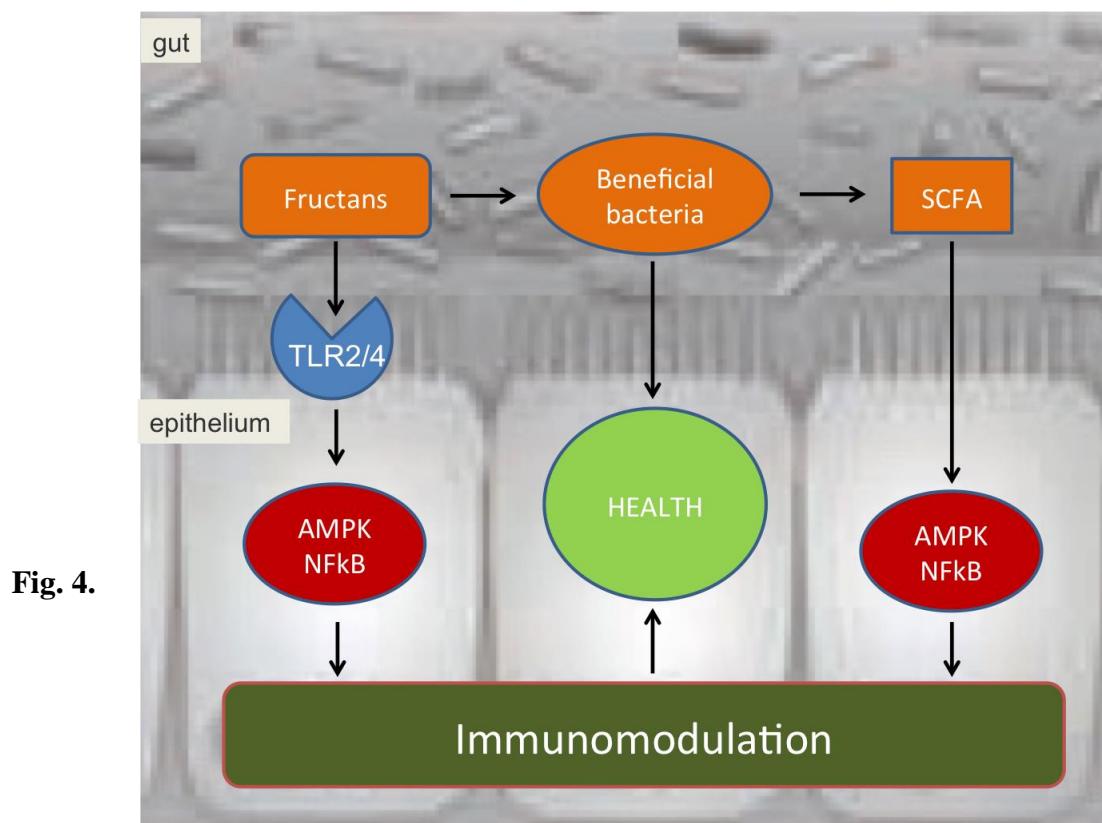
We refer to Incoll and Bonnett (1993) for more info on the occurrence of the different fructan types in (food) plants [53]. While plants need at least two distinct enzymes to catalyse priming and elongation, microbial fructan biosynthesis typically occurs by a single fructosyltransferase activity for both reactions, creating either levan- type fructans by levansucrases or inulin-type fructans by inulosucrases [54].

The best known plant-derived fructans in the food industry are inulin, oligofructose and fructo-oligosaccharides (FOS) because of their well-recognized prebiotic and health-improving effects [55]. Inulin is usually derived from chicory roots, and their partial hydrolysis yields oligofructose, which is also commonly referred to as “FOS”, but it has a plant origin. Fungal β -fructosidase activity on sucrose leads to the synthesis of FOS [56]. Fructans in human diets originate from wheat (*Triticum aestivum*), rye (*Secale cereale*), oat (*Avena sativa*), barley (*Hordeum vulgare*), leek (*Allium ampeloprasum*), Belgian endives (*Cichorium intybus*), lettuce (*Lactuca sativa*), salsify (*Scorzonera hispanica*), onion (*Allium cepa*), garlic (*Allium sativum*), globe artichoke (*Cynara scolymus*) and asparagus (*Asparagus officinalis*) [57]. The latter four species are extensively used in medicine with reported immunomodulatory and antiviral properties [57]. Wheat is by far the most important fructan source in the Western diet, as it accounts for approximately 70% of the daily fructan intake in the American and Western Europe diets [58]. Therefore, research is now focusing more on the metabolism and food applications of cereal fructans. Branched Agave-derived fructans may have similar properties and applications as cereal fructans [59].

Contrary to fructose, fructans (i) are not digested by the human upper gastrointestinal tract [50], (ii) induce satiety-sensing in food consumption and (iii) counteract lipogenesis in the liver [30]. How can inulin-type fructans in our diet

contribute to sustain health and overall well-being? They may act by selective stimulation of beneficial bacteria (e.g. *Lactobacilli* and *Bifidobacteria*) [60], relieve of constipation [61], lowering of blood glucose levels [62], improved mineral uptake [63], reduction in blood serum triacylglycerol levels [64], reduction of colon pH [56], increased production of SCFAs [56], reduced risk of colon cancer [65], stimulation of the immune system [66] and growth inhibition of pathogenic microorganisms [67]. We refer to our previous lengthy reviews for more elaborated descriptions and discussions on possible underlying mechanisms [55, 62]. Although dietary fructans generally mediate positive effects, it should be noted that doses exceeding 20 g/day may lead to abdominal pains and flatulence [68].

Historically, the positive effects of fructans on human health have been explained through indirect mechanisms via their positive influence on intestinal microflora [57] (Fig. 4). However, this view is now rapidly changing in favour of more direct mechanisms (Fig. 4).



Simplified model depicting direct and indirect effects of fructans at the gut interphase

Dietary fructans stimulate the growth of beneficial bacteria (*Bifidobacteria*, *Lactobacilli*) that ferment fructans to SCFA and gases (e.g. H_2) in the gut. After their uptake in gut epithelium cells, SCFA trigger AMPK/NFkB and other

signaling pathways leading to local or systemic (e.g. the liver) immunomodulatory effects. Dietary fructans (and other carbohydrates) may also directly stimulate TLR2/TLR4 signaling, linked to similar downstream signaling pathways and immunomodulatory effects promoting health. Additionally, beneficial bacteria produce a number of compounds that, after uptake in to the gut epithelium, promote overall health and well-being. AMPK: AMP-activated kinase; NF- κ B: nuclear factor kappa-light-chain-enhancer of activated B cells; SCFA: short chain fatty acids; TLR: Toll-Like Receptor.

In plants, fructans and other organic compounds occurring at elevated concentrations are more and more recognized as “antioxidants” that can be involved in the scavenging of hydroxyl radicals, contributing to cellular ROS homeostasis [69]. Moreover, similar processes might occur in foods and at the gut interphase in animals and humans [70]. Therefore, fructans may be useful in disease prevention by reducing ROS levels. It is well-known that an array of gut diseases are associated with ROS dynamics [70]. The antioxidant capacity of inulin was recently confirmed in *ex vivo* experiments [71].

Defense in plants relies on innate immunity responses, in contrast to higher vertebrates that rely both on innate and adaptive immunity [59]. The importance of the role of autophagy in innate and adaptive immunity is highlighted by the association of defects in autophagy with neurodegeneration, aging, cancer, metabolic syndrome and inflammatory disorders such as Crohn's disease [72]. It was recently proposed that fructans and other sugars may act as immunomodulatory signals in all eukaryotes [73]. The basis of this idea comes from the finding that inulin-type fructans trigger Toll-Like-Receptor (TLR2, TLR4) signaling pathways, intimately linked to Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF- κ B) and AMP-activated kinase (AMPK) signaling pathways [57] (Fig. 4). AMPK is an energy-sensing protein

kinase complex that monitors the metabolic status to maintain energy homeostasis [74]. Nutrient starvation leads to increased AMPK activity that stimulates autophagy, while mammalian target of rapamycin complex 1 (mTORC1) has the opposite effect [75]. Intriguingly, both inulin-type fructans and its fermentation products (e.g. butyrate) stimulate AMPK signaling [76] that stimulates autophagy. Whether inulin binds to membrane receptors or rather stimulates endocytotic (and autophagic) mechanisms, perhaps mediating its own uptake [77], remains a matter of debate.

Intriguingly, besides fructans, exogenously applied disaccharides sucrose and trehalose are emerging as sweet immunity agents in plants, stimulating stress responses. Similar to extracellular ATP, these sugars may be perceived as Damage Associated Molecular Patterns (DAMPs) [70]. Sucrose was reported to stimulate endocytosis and autophagy [78] Trehalose also stimulates autophagy [79]. In the context of neurodegenerative diseases, autophagy is proposed to remove the initial damaged mitochondria and aggregated proteins, leading to an effective antioxidative strategy, counteracting further neurodegeneration [80]. Thus, autophagy seems to emerge as an essential cell biological process in eukaryotic cells, and a central component of integrated stress responses, preventing and counteracting numerous diseases [73]. While fructans, sucrose and trehalose stimulate autophagy, fructose shows the opposite effect. Regardless of the need to further unravel the complexity of all the above-mentioned pathways, there is a general consensus within the scientific community that fructans are to be considered as functional foods and health-promoting compounds, as explained in more detail in our previous reviews [50, 57].

Conclusion

Both fructose and fructans are natural products available and assimilated through fruits and vegetables, and both are currently used as sweeteners. Although fructose and fructans are related and often confused by non-experts, they are absorbed and metabolized in a different way, and their physiological effects in the human body are entirely different, for instance with respect to their prebiotic capacities and their differential effects on AMPK signaling and autophagy, associated with possible disease prevention. According to the data collected so far we conclude that fructans benefit health as prebiotics,

antioxidants and immunomodulators, while fructose abuse (e.g. as sweeteners derived HFCS) may lead to opposite effects, although there remain huge uncertainties on the daily amounts of ingested fructose that could be considered harmful to human health, and this requires further investigations.

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